II. REMARKS

Claims 7-29 are presently pending in this application and stand variously rejected under 35 U.S.C. §§ 112 and 102. Applicants note with appreciation that the restriction requirement has been withdrawn and all claims were examined. Additionally, Applicants thank the Examiner for initialing and returning the 1449 forms to indicate that the references submitted by Applicants in their IDS have been considered.

Independent claim 7 has been amended to specify that the polynucleotide encodes a fragment of LT-A of at least 8 amino acids in length. Further, the fragment encoded by the claimed polynucleotide includes residue 72, numbered relative to SEQ ID NO:1, in which the wild-type alanine (A) residue of this sequence has been substituted with an arginine (R) residue. New claim 30 has been added indicating that the claimed polynucleotide includes amino acid sequences from any of SEQ ID NO:2 through 4. Support for the amendments can be found throughout the specification and claims as originally filed, for example, on page 5, lines 25-31, referencing Domenighini et al. (1995). This reference provides all of the wild-type sequences presented in Figure 12 and SEQ ID NOs:1 to 4. Accordingly, submitted herewith is Figure 12 and a corresponding Sequence Identification Listing.

Abstract

Submitted herewith, on a separate sheet, is an Abstract of the Disclosure as required by 37 CFR 1.72(b).

Drawings

Applicants acknowledge receipt of the Draftperson's Form and are submitting substitute drawings herewith.

Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 7-29 stand rejected under 35 U.S.C. § 112, first paragraph as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to use the claimed invention. In particular, the Office continues to assert that no particular LT-A having sequence with Ala or Arg in the indicated position 72 is included in the claims or enabled by the disclosure. (Office Action, page 3).

Applicants traverse.

The Office maintains that "no particular LT-A having sequence with Ala in the indicated position is included in the claims or is present in the specification." (Office Action, page 3). However, Applicants note that the test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation. *Ex parte Forman*, 230 USPQ 546 (P.T.O. Bd. Pat. App. & Int., 1986). Further, whenever the PTO makes a rejection for failure to teach and/or use the invention, the PTO must explain its reasons for the rejection and support the rejection with (i) acceptable evidence, or (ii) reasoning which contradicts the Applicants' claim: the reasoning must be supported by current literature as a whole and the PTO must prove the disclosure requires undue experimentation. *In re Marzocchi*, 439 F.2d 220, 223-24, 169 USPQ 367, 369-70 (CCPA, 1971).

Applying these rules to the present application, Applicants submit the specification clearly teaches one skilled in the art how to make and use an amino acid sequence of an LT-A subunit having an arginine residue at the position corresponding to Ala 72. In view of Applicant's disclosure and the state of the art, one of skill in the art could readily determine which residue of any LT-A polypeptide corresponds to the "Ala-72" of the LT-A protein defined on page 5, lines 25-30 (where Applicants note that the "Ala-72" residue is numbered in relation to one of the sequences disclosed in

Domenighini et al (page 5, lines 25-30) and that this amino acid residue is located on the second turn of the alpha-helix in LT-A and faces the NAD binding site). (see, *e.g.*, page 5, lines 29-31 of the specification). Techniques of comparing amino acid sequence and structure to determined which residue corresponds to Ala-72 as claimed are well within the ability of one having ordinary skill in the art. (see, *e.g.*, page 16, lines 22-32). Indeed, Figure 12, corresponding to Figures 1 and 2 of Domenighini, shows an alignment of various wild-type LT sequences. In light of this evidence, it is plain that it would not require undue experimentation to make and use the claimed Ala-72-Arg detoxified LT-A mutants. Furthermore, the Office has failed to present any evidence that determining the amino acid residue that corresponds to Ala-72 as defined in the specification requires undue experimentation. Accordingly, this rejection should be withdrawn.

Nonetheless, to expedite prosecution, Applicants have amended the claims to indicate that "Ala-72" is numbered relative to Figure 12 (SEQ ID NO:1). The specification plainly indicates that LT-A proteins from any *E. coli* strain can be used, so long as the amino acid residue to be mutated "corresponds to Ala-72" as numbered relative to SEQ ID NO:1 (a porcine LT-A sequence disclosed in Domenighini et al (Molec. Microbiol. (1995) 15:1165-1167). Ala-72 is located on the second turn of the alpha-helix in LT-A and faces the NAD binding site. (page 5, lines 28-31). One of skill in the art could readily determine the corresponding residue in any *E. coli* LT-A protein as described, for example, on page 5, lines 29-31 and page 16, lines 22-32 of the specification and as shown in Figure 12.

Moreover, contrary to the Office's assertion, the particular sequences of various LT-A proteins cited in the specification are in fact incorporated by reference, for example, on page 10, lines 28 to 30. Furthermore, as noted above, one of skill in the art could readily determine which residue in the various LT-A sequences disclosed (page 1, lines 27-30; page 2, lines 15-23, Figure 12) corresponds to Ala-72 of the claimed sequence. Even assuming, for the sake of argument only, that the sequence is "essential material,"

Applicants have now properly incorporated all of this material into the specification and claims as required by MPEP 608(p).

In view of the foregoing amendments and remarks, Applicants submit that the pending claims are fully enabled by the specification as filed and respectfully request that the rejection be withdrawn.

Written Description

The Office further asserts that the specification fails to convey to one of skill in the art that applicants were in possession of the claimed invention. In particular, it is alleged that the specification fails to convey that applicants were in possession of the claimed LT-A fragments.

In view of the foregoing amendments and following remarks, Applicants traverse the rejection and supporting remarks.

The written description requirement of section 112, first paragraph is satisfied if the specification, as filed, reasonably conveys to those skilled in the art that applicant was in possession of the claimed subject matter. *In re Ruschig*, 154 USPQ 118, 123 (CCPA 1967). Further, determining whether the written description requirement is satisfied requires reading the disclosure in light of the knowledge possessed by those skilled in the art. *In re Alton,* 37 USPQ2d 1578 (Fed. Cir. 1996). The present application plainly indicates that the inventors were in possession of the claimed invention at the time of filing. As noted in the Declaration by Dr. del Giudice, submitted in the parent application (Serial No. 09/297,171):

> 6. It is also my opinion that one working in this field would have expected functional fragments of the full-length LT-R72 to be immunogenic. The application indicates that such fragments must contain the Ala-72 residue and that these functional fragments are preferably at least 8 amino acids in length. (page 5, lines 14-15 and page 17, lines 10-19 of the application). Thus, is my further opinion that a scientist working in the field could have constructed, using conventional methods known in the art in combination

> with the teachings of the specification, immunogenic polypeptides which (1) were fragments of full-length LT-A; (2) contained sequence corresponding to the Ala-72 residue; and (3) were at least 8 amino acids in length. Furthermore, a scientist would have been readily able to test such constructs for immunogenicity; toxicity (*e.g.*, in the well known Y1 cell assay); and for their effectiveness as mucosal adjuvants (*e.g.*, following the guidelines of the application). Thus, I believe that the application as filed clearly conveys to a skilled artisan that the inventors were in possession of the claimed LT-A fragments at the time the application was filed.

Accordingly, without conceding the correctness of the Examiner's position and solely to advance prosecution, the claims have been amended herein to specify that the fragment is an immunologically effective detoxified fragment of an Ala-72-Arg mutant of at least 8 amino acids in length. As noted in the specification, for example on page 5, lines 14-15 "references to LT-A also encompass fragments of LT-A provided that the fragment contains Ala-72." Further, page 17, lines 10-19 indicate that amino acid sequences of at least 8 amino acids in length which is immunologically effective are included in the invention. It is well within the purview of the skilled artisan, in view of the teachings of the specification, to construct fragments of LT-A containing the Ala-72 mutation to Arg that are at least 8 amino acids in length. (see, *e.g.*, page 5, lines 14-15; page 17, lines 10-19 of the specification). These fragments could readily be tested for toxicity (*e.g.*, in the well known Y1 cell assay) and for their effectiveness as mucosal adjuvants (*e.g.*, following the guidelines of the specification). (see, *e.g.*, page 43. line 35 to page 44 line 21 for example of Y1 assay; and page 44, line 25 to page 46, line 33 for example of testing mucosal adjuvanticity). Thus, the specification clearly conveys to a skilled artisan that applicants were in possession of the precisely claimed LT-A fragments at the time the application was filed.

Therefore, the rejections under section 112, first paragraph are improper and Applicants respectfully request these rejections be withdrawn.

Rejections Under 35 U.S.C. § 102

Claims 7, 11, and 15 are rejected under 102(b) as allegedly anticipated by Domenighini et al. (WO 93/13202). It is alleged that Domenighini describes a polynucleotide encoding LT-A subunit having Ala at position 72 substituted with Arg residue and corresponding vector and expression cells. (Office Action, page 5).

Applicants traverse the rejection and supporting remarks.

In order to be an anticipatory reference, the single reference cited by the Office must disclose each and every element of the claims. *Hybritech v. Monoclonal Antibodies,* 231 USPQ 81 (Fed. Cir. 1986). Moreover, the single source must disclose all of the claimed elements arranged as in the claims. *See, e.g., Richardson v. Suzuki Motor Co.,* 9 USPQ2d 1913 (Fed. Cir. 1989).

Applicants have clearly established that the cited reference does not anticipate the pending claims. As previously and repeatedly noted, this reference does not describe or demonstrate that the claimed mutation results in a detoxified mutant. (See, *e.g.,* previous Response along with line 51 of page 46 which indicates that LT-R72 mutants are toxic). Moreover, with regard to polynucleotides encoding these detoxified mutants, Domenighini fails to disclose molecules encoding detoxified, fragments of at least 8 amino acids and in which the residue corresponding alanine at position 72 of SEQ ID NO:1 is substituted by arginine, as claimed by Applicants. Indeed, Domenighini discloses only polynucleotide 21-mers that encompass the Ala-72 position of SEQ ID NO:1. Such polynucleotides could, at best, encode only 7 amino acid long fragments. In sum, Domenighini fails to describe, demonstrate or suggest, at least, the following elements of the pending claims: (1) polynucleotides encoding 8 amino acid long fragments, (2) where the fragments include the R72 mutation; and (3) where the fragment is a detoxified mutant.

Accordingly, because Domenighini does not disclose key elements of the pending claims (*e.g.,* detoxified LT-R72 mutants and use of these mutants as mucosal adjuvants),

and because Applicants in the pending case have provided ample evidence distinguishing the claimed mutants from those of the prior art, the rejection under 102 is legally improper and should be withdrawn.

Rejections Under 35 U.S.C. § 103

Claims 7-29 are rejected under section 103 as allegedly obvious over EP145486. It is maintained that this reference teaches the compositions and vaccines comprising modified LT-A that includes an 8 amino acid fragment including the Ala-72 substituted residue. (Final Office Action, page 6, underlining residues 67-75 of the sequence shown).

Applicants traverse the rejection and supporting remarks.

The pending claims are directed to polynucleotides encoding detoxified fragments of LT-R72 mutants. Furthermore, these fragments include a mutation corresponding to position 72 (Alanine to Arginine) of SEQ ID NO:1. The sequence disclosed in EP 145486 includes an 18 amino acid residue leader sequence at positions 1-18. Thus, when SEQ ID NO:1 is aligned with the sequence presented in EP 145486, amino acid residue 72 (as claimed) is actually residue 89 of the cited sequence. As such, the fragment underlined by the Examiner in the rejection does not fall within the scope of the pending claims.

Indeed, a comparison of Figure 1 and Figure 2 in this reference indicates there is absolutely no change in the residue corresponding to position 72 of SEQ ID NO:1. In both wild-type and mutant, Ala remains Ala at this position. Nowhere does EP 145486 describe, demonstrate or suggest mutated LT sequences in which the particular residue numbered relative to SEQ ID NO:1 as recited in the claims is substituted. Nor does this reference teach or suggest use of any LT mutants as adjuvants. Therefore, the cited reference fails to teach or suggest the precisely claimed invention and, accordingly, withdrawal of the rejections based on this reference is respectfully requested.

III. CONCLUSION

In view of the foregoing, Applicant submits that the claims are now in condition for allowance and requests early notification to that effect.

Please direct all further communications regarding this application to:

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Version Showing Changes Made to Specification

On page 10, line 1, the following paragraph has been added:

--**Figure 12** (SEQ ID NOs:1 through 4) is an alignment of amino acid sequences of wild-type LT-A. SEQ ID NO:1 shows a wild-type sequence of a porcine LT. SEQ ID NO:2 shows a wild-type sequence of a human LT. SEQ ID NO:3 and SEQ ID NO:4 show wild-type sequences of two variants of human Type II LT, designated IIa and IIb, respectively. Dashes indicate identical residues as compared to SEQ ID NO:1. Gaps introduced to maximize alignments are shown by periods.--

Version Showing Changes Made to Claims

7. (Amended) A polynucleotide encoding [an immunogenic detoxified protein comprising the amino acid sequence of subunit A of an *E. coli* heat labile toxin (LT-A), or] an immunologically effective detoxified fragment of an *E. coli* heat labile toxin (LT-A), wherein said fragment is at least 8 amino acids in length and further wherein the said fragment comprises the amino acid residue corresponding to Ala-72 of SEQ ID NO:1 and wherein said residue is substituted with an arginine residue [wherein amino acid Ala-72 in said amino acid sequence is substituted with an arginine residue, or an immunological fragment thereof comprising the Ala-72 to arginine substitution and at least 5 amino acids].

30. (New) The polynucleotide of claim 7, wherein the fragment comprises amino acid residues of SEQ ID NO:2.

31. (New) The polynucleotide of claim 7, wherein the fragment comprises amino acid residues of SEQ ID NO:3.

32. (New) The polynucleotide of claim 7, wherein the fragment comprises amino acid residues of SEQ ID NO:4.

Currently Pending Claim Set

7. (Amended) A polynucleotide encoding an immunologically effective detoxified fragment of an *E. coli* heat labile toxin (LT-A), wherein said fragment is at least 8 amino acids in length and further wherein the said fragment comprises the amino acid residue corresponding to Ala-72 of SEQ ID NO:1 and wherein said residue is substituted with an arginine residue.

8. The polynucleotide of claim 7 further comprising a sequence encoding a second immunogenic antigen.

9. The polynucleotide of claim 8 wherein the second immunogenic antigen comprises a subunit B of an *E. coli* heat labile toxin (LT-B).

10. The polynucleotide of claim 9, wherein the LT-A and LT-B are encoded in a polycistronic unit.

11. An expression vector comprising the polynucleotide of claim 7.

12. An expression vector comprising the polynucleotide of claim 8.

13. An expression vector comprising the polynucleotide of claim 9.

14. An expression vector comprising the polynucleotide of claim 10.

15. A host cell comprising the expression vector of claim 11.

16. A host cell comprising the expression vector of claim 12.

17. A host cell comprising the expression vector of claim 13.

18. A host cell comprising the expression vector of claim 14.

19. The host cell of claim 15, wherein the host cell is selected from the group consisting of a bacterium, a mammalian cell, a baculovirus, an insect cell and a yeast cell.

20. The host cell of claim 19, wherein the host cell is *E. coli.*

21. The host cell of claim 19, wherein the host cell is a mammalian cell.

22. The host cell of claim 19, wherein the host cell is an insect cell.

23. The host cell of claim 19, wherein the host cell is a yeast cell.

24. The host cell of claim 19, wherein the host cell produces the amino acid sequence intracellularly.

25. The host cell of claim 19, wherein the host cell secretes the amino acid sequence.

26. The *E. coli* host cell of claim 19, wherein the host cell is mutated to produce a phenotype lacking wild type LT-A.

27. A method of producing a recombinant protein comprising:

(a) providing a population of host cells according to claim 15; and

(b) culturing said population of cells under conditions whereby the LT-A or fragment thereof encoded by the polynucleotide in said expression vector is expressed.

28. A method of producing a recombinant protein comprising:

(a) providing a population of host cells according to claim 17; and

(b) culturing said population of cells under conditions whereby the LT-A or fragment thereof and the LT-B encoded by the polynucleotide in said expression vector is expressed.

29. A method of producing a recombinant protein comprising:

(a) providing a population of host cells according to claim 26; and

(b) culturing said population of cells under conditions whereby the LT-A or fragment thereof encoded by the polynucleotide in said expression vector is expressed.

30. (New) The polynucleotide of claim 7, wherein the fragment comprises amino acid residues of SEQ ID NO:2.

31. (New) The polynucleotide of claim 7, wherein the fragment comprises amino acid residues of SEQ ID NO:3.

32. (New) The polynucleotide of claim 7, wherein the fragment comprises amino acid residues of SEQ ID NO:4.